REVIEWS AND ANALYSES

Microbial-Mediated Reduction of Perchlorate in Groundwater

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ABSTRACT

Perchlorate has been widely used as a propellant in solid rocket fuel, and has recently been identified as a contaminant in both groundwater and surface waters. Perchlorate is recognized by the U.S. Environmental Protection Agency (USEPA) as a potential health risk, and the State of California has set a drinking water action level of 18 μg L^{-1}. Incidents of groundwater contamination have been associated with industrial sites in California and Nevada that have been involved in the manufacturing or testing of solid rocket propellants. Microorganisms have been shown to be capable of reducing perchlorate (ClO_4^-) to chloride (Cl^-) and oxygen, thus transforming perchlorate into innocuous end-products. Bioreactor processes for the remediation of perchlorate contaminated wastewater have previously been established. However, these systems were optimized for perchlorate concentrations in the grams per liter range, while groundwater contamination can be a million-fold lower but still exceed the water quality action level. This literature review will focus on microbial-mediated perchlorate reduction, and discuss issues of importance to the remediation of perchlorate-contaminated groundwater.

Perchlorate (ClO_4^-) is an oxyanion that has been used extensively in the chemical and aerospace industries because it can act as a strong oxidizing agent. The mishandling of perchlorate at aerospace-related industrial sites is the likely source of perchlorate that has recently been discovered in surface and groundwaters. The persistence of perchlorate in the environment and its toxicity to humans at sufficient concentrations has raised concern over drinking water quality and possible environmental impacts. Perchlorate is not currently regulated under the Safe Drinking Water Act, although the California Department of Health Services has established an action level for perchlorate in drinking water of 18 μg L^{-1}. The basis of the action level is an evaluation of toxicity data by the USEPA (details are available from the California Department of Health Services homepage at www.dhs.cahwnet.gov). Perchlorate has been found in certain drinking water wells in California at concentrations that exceed the action level. In response, the California Department of Health Services has advised that water from these wells should not be used as a source of drinking water.

The development of effective and efficient strategies for the remediation of perchlorate in groundwater is an area of intense interest. Remediation strategies for the removal of perchlorate based on adsorption by activated C have not proven to be effective due to rapid saturation of perchlorate adsorption sites. Other advanced procedures for the removal of perchlorate include reverse osmosis and ion exchange, which are very expensive. An alternative strategy is the reduction of perchlorate by a biological means. Reduction of perchlorate provides an attractive remediation strategy because complete reduction can transform the compound into innocuous end-products, namely, chloride and oxygen. Biological-mediated reduction has been used successfully in the remediation of other toxic compounds, including chromate Cr(VI) (Losi et al., 1994a, b), selenate and selenite (SeO_4^{2-} and SeO_3^{2-}) (Losi and Frankenberger, 1997), as well as in the removal of nitrate (NO_3) and nitrite (NO_2) from treated wastewater (Mateju et al., 1992). This review will evaluate microbial-mediated perchlorate reduction as a biological remediation strategy.

PERCHLORATE IN THE ENVIRONMENT

The primary use of perchlorates is in solid rocket fuel. Solid rocket propellants can contain as much as 70% ammonium (NH_4) perchlorate as finely ground, crystalline particles dispersed within a polymer matrix. The polymer-binding matrix, with added aluminum, act as the fuel that reacts with the oxidizer. Ammonium perchlorate is also used in the production of explosives, pyrotechnics, and blasting formulations. Other perchlorate forms are used in dry batteries and oxygen-generating systems. The U.S. domestic capacity of NH_4 perchlorate is estimated to be more than 30 000 t /yr, with actual production depending on demand (Kirk-Othmer Encyclopedia of Chemical Technology, 1996). Wastewater generated from the manufacturing, maintenance, and testing of solid rocket propellants can contain NH_4 perchlorate concentrations in the grams per liter range.

The problem of perchlorate contamination of water supplies is a recently emerging issue. Much of the information presented below concerning the occurrence of perchlorate contamination is accessible from the California Department of Health Services home page at www.dhs.cahwnet.gov. There are several case histories of perchlorate in groundwater at industrial sites operated by aerospace companies. The Aerojet General Corporation, near Sacramento, CA, operated a facility primarily involved in the development and testing of rocket fuels. Aerojet has been treating water from a shallow aquifer to remove volatile organic chemicals, such as TCE, and has found this water also contains approximately 8 mg L^{-1} perchlorate. Aerojet is in the development stages of testing a granulated active C/fluidized bed reactor system to remove perchlorate from the water. In Nevada, sampling of wells at a current and former site of NH_4 perchlorate manufacturing were reported to have perchlorate concentrations >600 mg L^{-1} (Las Vegas Sun, see homepage at www.lasvegassun.com).

Perchlorate contamination has been shown to be a wide-

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Abbreviations: BOD, biochemical oxygen demand

spread problem. A survey reported by the California Department of Health Services revealed that of 53 wells tested in northern California, and 449 wells tested in central and southern California, perchlorate levels exceeded the 18 μg L^{-1} action level in 8 and 25 wells, respectively. Testing of drinking water wells in Riverside and San Bernardino Counties (California) revealed some wells to contain as much as 216 μg L^{-1} perchlorate, resulting in the closure of nine wells. Major surface water systems have also been shown to contain perchlorate, as indicated by levels as high 165 μg L^{-1} found in some areas of Lake Mead (Colorado River) and 8 μg L^{-1} in river water south of the lake.

TOXICITY OF PERCHLORATE

Medical knowledge concerning perchlorate toxicity stems from its use as an antithyroid agent in the treatment of Graves' disease. Graves' disease is a common cause of hyperthyroidism with a prevalence estimated to be one to two cases per 1000 yr^{-1} (Wilson and Foster, 1992). Perchlorate had been used in the treatment of hyperthyroidism because it can act to reduce thyroid iodide transport, thus decreasing the production of thyroid hormones. Due to its potential toxicity, perchlorate has been replaced by superior drug treatments. There is little information concerning the toxicity of perchlorate to humans. Adverse effects on the thyroid have been reported at doses that would correspond to drinking water concentrations of 49 mg L^{-1} (see California Department of Health Services homepage for more details). Based on available toxicity data, a water quality action level has been set at 18 μg L^{-1} by California's Department of Health Services. The health effects of long-term, low-level exposure are currently being investigated.

OTHER CHLOROXYANIONS

There is more information available concerning the microbial reduction of chlorate (ClO_3^-) than of perchlorate. The major use of sodium chlorate is in the generation of chlorine dioxide for bleaching of wood pulp. Chlorine dioxide cannot be transported and is therefore generated on site by pulp producers. Chemical bleaching alone was estimated to use 921 000 t yr^{-1} in 1990 (Encyclopedia of Chemical Technology, 1996), which is 30 times greater than perchlorate production. Some of the chlorine dioxide used in the bleaching process is released as chlorate, and it has been reported that chlorate concentrations in pulp mill effluents can be as high as 53 mg L^{-1} (Rosemarin et al., 1994). Chlorates are also used in the manufacturing of perchlorates, and as defoliants and desiccants in the production of cotton (*Gossypium herbaceum*) and soybean (*Glycine max* L.), respectively. Chlorates can also be released into the environment from the use of chlorine dioxide and hypochlorite as disinfectants in water treatment.

The environmental impact of chlorate was reviewed by van Wijk and Hutchinson (1995) who reported that most aquatic organisms can tolerate high concentrations of chlorate (>100 mg L^{-1}). The exception is the macro brown algae that were sensitive to 0.1 mg L^{-1} chlorate. The toxicity of chlorate in pulp mill effluents to macro brown algae (e.g., *Fucus* spp.) in the Baltic Sea has been investigated (Lehtinen et al., 1988; Rosemarin et al., 1994). The mechanism of toxicity is not completely understood although it is believed that chlorate itself is not toxic, but rather it is transformed into a more toxic metabolite, chlorite (ClO_2^-). Many plants, algae, and bacteria can transform chlorate to chlorite by assimilative and dissimilative NO_3^- reduction enzyme systems. Two features of the NO_3-reductase enzyme system are important in terms of chlorate toxicity. Firstly, NO_3^- reductase is an inducible enzyme, and secondly, the NO_3-reductase enzymes in certain species have a much higher affinity for NO_3^- than chlorate (van Wijk and Hutchinson, 1995; Balch, 1987). Therefore, NO_3 concentration is a modulator of chlorate toxicity. In environments where NO_3^- is limiting, or when chlorate levels are much greater than NO_3^- levels, the toxicity of chlorate can be enhanced.

Although the transformation of chlorate into chlorite is thought to be a key factor in chlorate toxicity, the difference between sensitive and resistant organisms remains unexplained. It was hypothesized by van Wijk and Hutchinson (1995) that nonsensitive species are capable of reducing chlorate all the way to chloride, without the formation of a toxic intermediate.

The activation of chlorate toxicity has been used in the selection of NO_3^- reductase-deficient bacteria from anaerobic cultures. The mutants are resistant to chlorate because they lack the NO_3^- reductase that activates chlorate through its reduction to chlorite. However, other bacteria have shown the capability to use chlorate as a final electron acceptor during the anaerobic respiration of organic compounds (as will be discussed in more detail in a later section). The complete reduction of chlorate to chloride and oxygen under anaerobic growth conditions represents a biotreatment strategy that has been successfully applied to the remediation of effluent produced by the pulp and paper industry (Malmqvist et al., 1991; Malmqvist and Welander; 1994).

MICROBIAL-MEDIATED REDUCTION OF PERCHLORATE

Microbial-mediated reduction of perchlorate can be viewed as a promising strategy for the remediation of contaminated water. Microbial reduction of oxyanions can occur as the result of anaerobic respiration. Microbial respiration couples the oxidation of an organic substrate, such as glucose or acetate, to the reduction of a final electron acceptor, usually oxygen. Under anaerobic conditions, the oxidation of organic compounds requires the use of an alternative electron acceptor in place of oxygen, such as NO_3^-, Mn(IV), Fe(III), or SO_4^{2-}. Bacteria capable of anaerobic respiration are common to soil and sediments where anaerobic conditions are prevalent and natural sources of alternate electron acceptors are common. As a highly oxidized compound (+7 oxidation state), perchlorate has a high potential for utilization as an alternate electron acceptor. Rikken et al. (1996) provided equations for the stoichiometric reaction of acetate with oxygen and perchlorate as electron acceptors.

$$CH_3COO^- + 2O_2 \rightarrow 2\ HCO_3^- + H^+$$

$$\Delta G^{o\prime} = -844$$

$$CH_3COO^- + ClO_4^- \rightarrow 2\ HCO_3^- + H^+ + Cl^-$$

$$\Delta G^{o\prime} = -966$$

Comparing the Gibbs free-energy changes ($\Delta G^{o\prime}$, KJ mol acetate^{-1}) it is evident that perchlorate reduction is energetically favorable.

There are several reports of mixed bacterial cultures capable of reducing perchlorate under anaerobic growth conditions. These bacteria were enriched from samples of sewage sludge in media containing various sources of organic C, mineral nutrients, and high concentrations of perchlorate (500–1000 mg L^{-1}, depending on the study). Acetate was shown to serve as the sole C source for the reduction of (per)chlorate (Rikken et al., 1996; van Ginkel et al., 1995; Malmqvist et al.,

1994; Korenkov et al., 1976). Using an acetate medium, Rikken et al. (1996) reported that 800 mg L^{-1} perchlorate was completely reduced after 9 d. In contrast, Attaway and Smith (1993) used a mixture of nutrient broth and yeast extract for the enrichment of a perchlorate-reducing culture, and reported reduction of 1000 mg L^{-1} perchlorate after approximately 2 d. They determined that perchlorate reduction occurred between pH 6.6 and 7.5, with an optimum at 7.1, and a temperature range between 25 to 42°C, with an optimum at 42°C.

Perchlorate-reducing bacteria include *Vibrio dechloraticus* Cuznesove B-1168 (Korenkov et al., 1976; Romaneko et al., 1976), *Wolinella succinogenes* HAP-1 (Wallace et al., 1996), and a proteobacteria, strain GR-1, described by Rikken et al. (1996). Bacteria capable of reducing chlorate include *Ideonella dechloratans* (Malmqvist et al., 1994) and an *Acinetobacter* sp. (Stepanyuk et al., 1992).

The reduction of (per)chlorate under anaerobic growth conditions has been shown to be directly proportional to the release of chloride. This would indicate that perchlorate can be completely reduced to chloride and oxygen (Rikken et al., 1996; van Ginkel et al., 1995; Attaway and Smith, 1993; Malmqvist et al., 1991). The reduction of (per)chlorate was also accompanied by biomass production, indicating that the microbial reduction of (per)chlorate is coupled to energy-yielding reactions (Rikken et al., 1996; Malmqvist et al., 1991). Rikken et al. (1996) proposed the following pathway for the reduction of perchlorate.

$$\underset{\text{perchlorate}}{ClO_4^-} \rightarrow \underset{\text{chlorate}}{ClO_3^-} \rightarrow \underset{\text{chlorite}}{ClO_2^-} \rightarrow \underset{\text{chloride}}{Cl^-} + O_2$$

They reported that anaerobic oxidation of an organic C source (acetate) was coupled to the reduction of perchlorate to chlorate and of chlorate to chlorite. However, the bacterium did not derive physiological useful energy from the reduction of chlorite to chloride. Chlorite can be inhibitory to microbial activity, and the transformation of chlorite to chloride is believed to be an enzymatic detoxification mechanism that protects the cell and allows the bacterium to use perchlorate and chlorate as electron acceptors. The purification and characterization of the chlorite-reducing enzyme has been reported (Wallace et al., 1995; van Ginkel et al., 1996). A perchlorate-reducing proteobacteria was found to contain a heme iron enzyme that catalyzed chlorite to chloride and oxygen (van Ginkel et al., 1996). The enzyme, chlorite dismutase, displayed maximal activity at pH 6.0 and 30°C, and was also found to obey Michaelis-Menton kinetics. The calculated kinetic parameters, V_{max} of 2200 U mg^{-1} (protein), where one unit (U) of activity is the amount of enzyme required to convert 1 μmol of chlorite/min, and K_{max} of 170 μM, indicated that the dismutase is efficient in removing chlorite. Strain *W. succinogenes* HAP-1 demonstrates a chlorite dismutase activity that is at least 1000-fold greater than the perchlorate or chlorate-reductase activities. Therefore, the chlorite produced during (per)-chlorate reduction should not accumulate to toxic levels (William Wallace, 1998, personal communication).

The (per)chlorate-reducing bacteria currently described are capable of using NO_3^- as an electron acceptor in anaerobic respiration. It has been suggested that the enzymatic activity supporting the reduction of chloroxyanions is linked to the enzymes involved in NO_3^- reduction, namely, NO_3^- reductases (Romaneko et al., 1976). However, not all denitrifying bacteria are capable of growth with chlorate as an electron acceptor, and there is evidence for a specific (per)chlorate reductase enzyme system that is separate from NO_3^- reduction. In the case of *W. succinogenes* HAP-1 (Wallace et al., 1996), the presence of NO_3^- did not interfere with perchlorate reduction. This would suggest that the enzymes involved in perchlorate reduction were not necessarily the same as those involved in NO_3^- reduction. The chlorate-reducing isolate, *I. dechloratans*, described by Malmqvist et al. (1994), lost its ability to reduce NO_3^- when grown for long periods on chlorate as the sole electron acceptor. Malmqvist et al. (1994) suggested that chlorate reduction is performed by a modified NO_3-reducing enzyme system, and that this could explain the occurrence of chlorate reducers even though chlorate has been present in the environment only as a result of human activity and only for a short period, from an evolutionary perspective, for the development of an enzyme system for the utilization of chlorate as an electron acceptor in anaerobic respiration.

Oxygen is a major inhibitor of (per)chlorate reduction (van Ginkel et al., 1995), and exposure of an active perchlorate-reducing culture to air can immediately halt perchlorate reduction (Attaway and Smith, 1993). Attaway and Smith (1993) also noted that cultures which were actively reducing perchlorate could reach a stage in which perchlorate reduction ceased, and the medium would be in an oxidized state, as indicated by a change in color of a redox indicator, resazurin, from clear to pink. They reported that the oxidation of the medium was probably caused by the formation of transient chloride oxide metabolites, possibly chlorite or hypochlorite. Perchlorate reduction would resume with the addition of a reducing agent, cysteine hydrochloride, which reduced the resazurin from pink to clear. The need to maintain redox conditions in which resazurin is converted from pink to clear to sustain perchlorate reduction would indicate a redox potential requirement of less than −110 mV for perchlorate reduction.

The choice of a C substrate to act as an electron donor can be an important consideration. Acetate has been used successfully to support (per)chlorate reduction (Rikken et al., 1996; van Ginkel et al., 1995; Malmqvist et al., 1994; Korenkov et al., 1976). In contrast, Attaway and Smith (1993) enriched perchlorate-reducing bacteria using a nutrient broth/yeast extract mixture, and then tested a variety of C substrates to determine their suitability for support of perchlorate reduction. They noted that a wide variety of organic acids, including acetate and alcohols promoted growth without sustaining perchlorate reduction. Perchlorate reduction was only evident with the addition of protein-based C sources, including nutrient broth, peptone, yeast, and casamino acids. In a study using a chlorate-reducing mixed culture that had been enriched with acetate, van Ginkel et al. (1995) noted that a wide variety of organic substrates, including alcohols, carboxylic acids, and amino acids could be oxidized to support chlorate reduction. Fermentable substrates, such as glucose, lactose, carboxylmethyl cellulose, and starch, did not directly support chlorate reduction, although it was shown with glucose that the formation of fermentation products, acetic acid and formic acid, supported chlorate reduction.

BIOREACTOR SYSTEMS FOR PERCHLORATE REDUCTION

Several patents have described processes for the use of microbial-mediated reduction of perchlorate as a means to remediate industrial waste. A Yakovlev et al. (1973) patent describes the use of unaerated sewage sludge for the treatment of certain oxygen-containing inorganic chlorine and metal compounds, including perchlorate, chlorate, and chromate. Domestic sewage sludge was mixed with contaminated wastewater and placed in a large tank. In the absence of aeration, microbial utilization of organic material within the sludge rapidly depleted the available oxygen. Under anaerobic condi-

tions, the reduction of oxygen-containing inorganic compounds occurred with the oxidation of organic compounds. Following the anaerobic phase, a second stage in the process removed the sludge from the water by precipitation. It is important in this process to supply an excess quantity of organic material, as measured by biochemical oxygen demand (BOD), to ensure the creation of an anaerobic environment.

Several later patents have improved on this basic process, enhancing the rate and extent of perchlorate reduction, and enabling the treatment of higher concentrations of perchlorate in wastewater. Korenkov (1976) described a method of reducing perchlorate and chlorate under anaerobic conditions using the bacterium, *V. dechloraticans* Cuznesove B-1168. This organism is capable of reducing perchlorate and chlorates when grown anaerobically on acetate or ethanol as a C source (Romanenko et al., 1976). The authors reported reduction rates of perchlorate as high as 70 mg ClO_4^- h^{-1} g^{-1} biomass solids (dry wt.), and the ability to treat perchlorate concentrations as high as 3 m*M* (about 300 mg L^{-1}). A 1994 patent (Attaway et al., 1994) describes a process in which contaminated water is added to an anaerobic bioreactor and spiked with a mixed bacterial culture. The bacterial culture contains a specific bacterium, *W. succinogenes*, which was isolated from domestic sewage sludge for its ability to reduce very high concentrations (>7000 mg L^{-1}) of perchlorate (Attaway and Smith, 1993; Wallace et al., 1996). High protein organic nutrients were found to support perchlorate reduction, and the source of this oxidizable organic matter in the anaerobic bioreactor could be in the form of aged brewers yeast, cottonseed protein or whey powder. A second stage in the process removes nutrients and organic matter to improve the quality of the water for discharge. One advantage of this system is that it does not use sewage sludge, and therefore eliminates problems associated with the presence of pathogens. The bacterium isolated was capable of reducing perchlorate concentrations 26-fold greater than in previous reports, and was reported to have a specific perchlorate degradation rate of at least 1492 mg ClO_4^- h^{-1} g^{-1} biomass (dry wt.). Through the use of a specific isolate and by optimizing nutrient and environment conditions, the anaerobic reactor was capable of greater perchlorate reduction rates than previously reported.

POTENTIAL USE OF MICROBIA-MEDIATED REDUCTION IN THE REMEDIATION OF PERCHLORATE IN GROUNDWATER

The processes described above dealt with the remediation of perchlorate in the milligram per liter concentrations that are associated with wastewater generated from handling of rocket propellant in an industrial setting. Rikken et al. (1996), van Ginkel et al. (1995), and Attaway and Smith (1993) reported the stoichiometric conversion of perchlorate to chloride, indicating that complete reduction of perchlorate was occurring. However, the limits of detection of the analyses made were not stated. There have been no reports of biotreatment systems optimized for the reduction of perchlorate concentrations in the microgram per liter range; the level of contamination of current concern.

To be effective, a remediation system must ensure the reduction of perchlorate concentrations to less than the current action level of 18 μg L^{-1} and the complete reduction of perchlorate to chloride and oxygen. The requirements for perchlorate reduction includes anaerobic conditions, neutral pH, moderate to high temperatures, and a C source suitable for respiration. However, little attention has been given to the factors that may interfere with perchlorate reduction in a groundwater matrix.

One issue of concern is the presence of a variety of possible electron acceptors in groundwater. In the environment there can be a distinct sequence by which electron acceptors are used (see Table 1). The basis of this sequence is the "selection" by the microbial community of an electron acceptor that will maximize energy yield. A higher energy yield from the oxidation of a C substrate will bestow a competitive advantage to a particular population, such as NO_3^- reducers over sulfate reducers, until the available NO_3^- has been exhausted. The position of perchlorate in the sequence of dominant terminal electron-acceptors is not known.

Bacterial isolates capable of growth via (per)chlorate reduction have been shown to reduce NO_3^- (Attaway and Smith, 1993; Malmqvist et al., 1994; Rikken et al., 1996), manganese [Mn(IV)] (Rikken et al., 1996), and sulfate (Attaway and Smith, 1993). There have been limited and conflicting reports on the ability of these alternative electron acceptors to affect the rate of (per)chlorate reduction. Van Ginkel et al. (1995) found that the presence of NO_3^- competitively inhibited chlorate reduction, while Mn(IV), Fe(III), and sulfate had no influence. In contrast, Wallace et al. (1996) reported that *W. succinogenes* HAP-1 preferentially reduced perchlorate prior to reducing NO_3^-. Attaway and Smith (1993) also showed that the presence of NO_3^- and sulfate did not inhibit the rate of perchlorate reduction. However, they did find that the presence of chlorate competitively inhibited the reduction of perchlorate, and that complete inhibition of perchlorate reduction was evident in the presence of NO_3^- and chlorite, probably as a result of direct toxicity to the cell.

A second consideration that has not previously been addressed is the determination of a threshold concentration of perchlorate that will promote and sustain the growth of perchlorate reducers. Perchlorate concentrations may be too low to support the activity of perchlorate-reducers, and, therefore, not maintain a viable population. This condition may be exacerbated if NO_3^- levels are high relative to perchlorate levels, and the perchlorate reducers are in competition with denitrifiers for nutrients. Such a situation may create problems in the maintenance of a high density of perchlorate-reducing cells. One possible solution would be to pretreat the groundwater to remove NO_3^-. A second possible solution is to supplement the groundwater via chemical analog enrichment to support perchlorate reduction and maintain a perchlorate-reducing population that is competitive with denitrifiers. However, it will be necessary to ensure that chemical analog supplementation will promote the complete removal of all chloroxyanions.

Current efforts to remove selenium (Se) from agricultural drainage waters (Thompson-Eagle and Frankenberger, 1992) can provide valuable insights for the development of a perchlorate bioremediation strategy. Selenium is a trace metalloid found in the Central Valley of California with naturally Se-enriched soils. Intensive agriculture has resulted in the mobilization of Se into agricultural drainage. Excess Se in evapora-

Table 1. The sequence of utilization of electron acceptors most commonly found in groundwater (from Smith [1997] with permission).

Oxidized form	Reduced form	Concentration range generally found in groundwater
O_2	H_2O	0–0.4 m*M*
NO_3^-	N_2	0–20 m*M*
Mn^{4+}	Mn^{2+}	Very low
Fe^{3+}	Fe^{2+}	Very low
SO_4^{2-}	S^{2-}	0–15 m*M*
CO_2	CH_4	0–4 m*M*

tion ponds is toxic to wildlife, and can cause reproductive deformities. The common forms of Se in agricultural drainage are the oxyanions, selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}). Microbial-mediated reduction has the potential to remove selenium oxyanions in the form of elemental Se, which is a much less soluble and bioavailable form of Se. The effect of NO_3^- on selenate reduction has been an area of intensive study. Oremland et al. (1990) investigated the reduction of NO_3^-, selenate, and sulfate in evaporation pond sediment and found that selenate reduction primarily occurs near the surface of the sediment, above the zone of sulfate reduction, and that selenate is reduced in the same sediment zone as NO_3^-. However, not all NO_3-reducing bacteria can reduce selenate, and selenate reduction can be inhibited until all the available NO_3^- has been reduced (Steinberg and Oremland, 1990; Steinberg et al., 1992). Several different selenate-reducing bacteria have been isolated from Se-contaminated environments (DeMoll-Decker and Macy, 1993; Losi and Frankenberger, 1997), and have been shown to reduce selenate and NO_3^- simultaneously, and to reduce selenate all the way to elemental Se. In the case of *Thauera selenatis*, which can reduce selenate to selenite, the complete reduction of selenate to elemental Se is dependent on the presence of NO_3^- (DeMoll-Decker and Macy, 1993). The authors concluded that NO_2^- reductase, or a component of the NO_2^- respiratory system, is involved in the reduction of selenite to elemental Se while also reducing NO_2^-. Macy et al. (1993) optimized a biological reactor system for the anaerobic treatment of selenate-NO_3^- containing agricultural drainage water, and, with the addition of *T. selenatis*, reduced 350 to 450 μg L^{-1} Se to 5.4 to 3.6 μg L^{-1} Se.

The Se experience provides incentive for the isolation and characterization of perchlorate-reducing bacteria, and to investigate their ability to reduce perchlorate concentrations to the low microgram per liter range. More research is needed on specific issues related to perchlorate reduction, including the physiological capabilities of perchlorate-reducing isolates, the mechanism of perchlorate reduction, the selectivity of the isolate for perchlorate, the sequence of preference shown by bacterial populations for electron acceptors relative to perchlorate, and the threshold concentration of perchlorate that will maintain and promote the activity of the perchlorate-reducing bacterial population.

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